

**UNITED STATES AIR FORCE  
RESEARCH LABORATORY**

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**REPEATED DOSE SKIN IRRITATION  
STUDY ON JET FUELS - PRELIMINARY  
DOSE RANGE FINDING STUDY**

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## TECHNICAL REVIEW AND APPROVAL

**AFRL-HE-WP-TR-1999-0008**

The animal use described in this study was conducted in accordance with the principles stated in the "Guide for the Care and Use of Laboratory Animals", National Research Council, 1996, and the Animal Welfare Act of 1966, as amended.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

## FOR THE DIRECTOR



**STEPHEN R. CHANNEL**, Maj, USAF, BSC  
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## PREFACE

This is one of a series of technical reports describing results of the experimental laboratory programs conducted at the Operational Toxicology Branch under the ManTech Geo-Centers Joint Venture contract. This document serves as a preliminary report on the repeated dose skin irritation study on jet fuels. The research described in this report began in April 1998 and was completed in July 1998 under Department of the Air Force Contract No. F41624-96-C-9010. Maj Stephen Channel served as the Contracting Officer's Representative for the U.S. Air Force, Air Force Research Laboratory. Darol E. Dodd, Ph.D., served as Program Manager for ManTech Geo-Centers Joint Venture.

The animals used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals* prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, National Academy Press, 1996, and the Animal Welfare Act of 1966, as amended. The authors gratefully acknowledge the excellent technical assistance of Willie Malcomb, Jerry W. Nicholson, and Margaret A. Parish of ManTech Environmental, Wright-Patterson AFB, OH.

## TABLE OF CONTENTS

SECTION	PAGE
PREFACE .....	iii
TABLE OF CONTENTS.....	iv
LIST OF TABLES.....	v
LIST OF FIGURES .....	vi
INTRODUCTION .....	1
Study Objective.....	1
MATERIALS AND METHODS.....	2
Test Materials.....	2
Laboratory Animals and Animal Husbandry .....	2
Experimental Design.....	2
General Procedures and Experimental Evaluation .....	3
Clinical Observations and Body Weights.....	3
Termination and Gross Necropsy .....	3
Histopathology and Methods of Assessment.....	4
RESULTS AND COMMENTS.....	6
Test Material Analysis .....	6
Addressing Initial Study Objectives .....	7
Clinical Observations and Body Weights.....	7
Histopathology .....	8
Recommendations for Definitive Study.....	10
REFERENCES .....	11
APPENDIX A. Individual animal histopathology assessment sheets .....	12

## LIST OF TABLES

TABLE		PAGE
1	Group Assignment and Treatment.....	2
2	Skin Samples.....	4
3	Fuel Specifications Results.....	6
4	Average Severity Scores of Combined Skin Lesions in Treated and Untreated Samples .....	8
5	Frequency of Skin Lesions in Treated and Untreated Samples .....	9
6	Average Severity Scores of Skin Lesions in Treated and Untreated Samples .....	10

## LIST OF FIGURES

FIGURE	PAGE
1 Neck Collar .....	3



# **REPEATED DOSE SKIN IRRITATION STUDY ON JET FUELS – PRELIMINARY DOSE RANGE FINDING STUDY**

## **INTRODUCTION**

JP-8 is the battlefield fuel for DoD and NATO countries. Its use is projected well beyond 2025, with changes to meet new weapons systems requirements through the use of additive packages to the parent JP-8 fuel. Questions have been raised about the human health implications of occupational exposures to JP-8, as compared to the phased out JP-4. Populations most likely to have repeated skin exposure to JP-8 are those in refueling operations, bulk storage and distribution, jet engine repair, and fuel cell maintenance and repair. Clinical observations indicate that skin contact results in acute redness and itching or dermatitis. No scientific information is available on the effect of repeated skin contact with JP-4 or JP-8.

### **Study Objective**

The initial objectives of this preliminary investigation were to 1) define the "dose" by determining the appropriate volume of test substance to be applied, the frequency (once or twice daily), and the specific surface area (cm<sup>2</sup>) for the site of test substance application, 2) observe and record hair growth following hair clipping of the application site and decide the frequency of clipping needed during the definitive study, 3) determine if normal animal holding restraint procedures are adequate for careful hair clipping procedures, and 4) determine the stress on the experimental animal that might be associated with the continuous wearing of neck collars.

An additional benefit of this study was the opportunity to preview the nature and severity of lesions to be encountered in the definitive study. This report presents the dermal lesions observed in the pilot study and demonstrates application of an established system (1) of evaluating and scoring the lesions. This effort thus formalized the proposed approach to evaluation of the skin samples collected in the definitive study and gave the investigators a method by which to semi-quantify and thus more objectively analyze the data.

## MATERIALS AND METHODS

### Test Materials

The test substances, JP-4 and JP-8 were supplied by the AFRL, Propulsion Directorate, Fuels Branch. Pertinent military specifications (MIL-SPEC) are MIL-T-83133D, 29 Jan 92 (JP-8) and MIL-PRF-5624S, 22 Nov 96 (JP-4). The test substances were analyzed for certain specification tests prior to the initiation of the study. Additional tests were conducted after the study in order to confirm the fuels still met the specification (see Results section). Test substances were stored in a flammable liquid storage cabinet under ambient conditions.

### Laboratory Animals and Animal Husbandry

Thirteen male Fischer rats [CDF®(F-344)/CrIBR], Charles River Laboratories, Wilmington, MA, weighing between 200 and 265 grams, were used. Animals arrived on two separate occasions (7 April 1998 and 30 June 1998) since the investigation was preliminary in scope and strict scheduling was not required. Routine animal husbandry procedures were performed by AFRL/HEST personnel using Standard Operating Procedures for rodents. The animals used in this study were handled in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, National Academy Press, 1996, and the Animal Welfare Act of 1966, as amended. The rats were housed in plastic shoe-box cages with bedding (one/cage). A 12-hr light/dark cycle was provided. Temperatures were maintained between 18 and 26°C, and relative humidity was maintained between 30 and 70%. Food (Formulab Rodent Diet, PMI Feeds, Inc., St. Louis, MO) and water were available *ad libitum*.

### Experimental Design

Group assignment and treatment with the test materials are given in Table 1. There were no control animals, but untreated dermal sites served as control areas (details below).

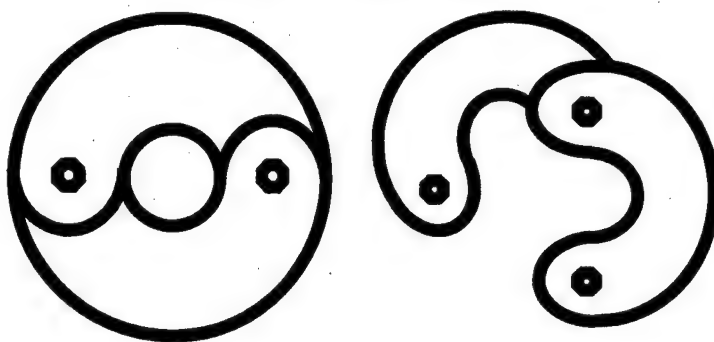
**Table 1. Group Assignment and Treatment**

Group Identification	Number of Rats	Treatment
JP-4 once daily	3	Dermal application of JP-4 once daily for 7 days
JP-4 twice daily	3	Dermal application of JP-4 twice daily for 7 days
JP-8 once daily	3	Dermal application of JP-8 once daily for 7 days
JP-8 twice daily	4	Dermal application of JP-8 twice daily for 7 days

## General Procedures and Experimental Evaluations

Each test substance was applied (neat) either once (a.m.) or twice (a.m. and p.m., about 4 to 5 hours apart) daily to the skin of experimental animals for a period of 7 days. The site of application was not wrapped, but animals wore neck collars to avoid grooming of the dosing area. The neck collars were custom-made and fabricated from approximately 3/16" thick rounded-off plastic (Figure 1). Two, approximately 1" wide, half crescents were riveted together at one end, so that the diameter of the hole formed in the center was adjusted by moving the half crescents inward and outward. The other end of the half crescents was fastened with a screw and bolt that, upon fastening, acts similar to a screw clamp to keep the collar in place once it was wrapped around the animal's neck.

Figure 1. Neck Collar



An area of 2.5 cm x 5 cm (12.5 cm<sup>2</sup>) was chosen as an appropriate surface area for dosing laboratory rats (see Results section). This area was measured on the dorsal side of the rat, using the midline as one of the 5 cm sides, and outlined using a felt tip marker. For each test substance, 0.156 mL was dispensed once or twice daily onto the 12.5 cm<sup>2</sup> surface area. A hand-held pipetter was used to draw the test substance from its container. During each application, the technician carefully dispensed the test substance as evenly as possible over the entire surface area. The skin at the site of test substance application was not abraded. Careful hair clipping procedures were carried out to avoid skin irritation. One person did hair clipping to standardize the process.

On each animal, an anatomically comparable, non-dosed area of skin was used for control. This untreated area was on the dorsal side of the rat, immediately opposite of the midline that served as a border for the treated area.

### Clinical Observations and Body Weights

The degree of skin irritation was observed each morning prior to application. At this time, animals were also observed for signs of stress (due to the wearing of neck collars), health status, and hair growth (at the site of dosing). Scoring the skin sites (treated vs. untreated) for signs of irritation followed the "standard procedure for evaluation of skin reactions" described by Draize and coworkers (2) and recommended by most regulatory authorities (3) in their guidelines on the conduct of animal tests for skin irritation. Animals weights were determined at various times throughout the 7-day study. Animals were weighed without removing neck collars. Collar weights were not subtracted from the body weight data.

### Termination and Gross Necropsy

At study termination, animals were subjected to gross necropsy following CO<sub>2</sub> inhalation overdose.

Samples of skin from treated and untreated areas were fixed in 10% buffered formalin for at least 24 hours. Following fixation tissues were processed using standard protocols for paraffin embedding, sectioning, mounting, and hematoxylin and eosin staining. For one group of study animals (JP-4 twice daily), the liver, kidneys, and lungs were trimmed, weighed wet, and preserved in buffered formalin.

### **Histopathology and Methods of Assessment**

Samples of skin from individual animals were collected for histopathological evaluation (Table 2).

**Table 2. Skin Samples**

		Test Substance and Frequency of Application			
		JP-4		JP-8	
	Controls	1x/day	2x/day	1x/day	2x/day
Number of Samples	5*	3	3	3	4

\*Control samples came from untreated side of 5 treated animals.

Microscopic slides were assessed in random order and graded according to the changes noted on the individual animal assessment sheets (Appendix A). The grading of lesions were scored as follows.

**Erosion:** 0=normal, 1=focus limited to region above a dermal papilla, 2=region over several hair follicles or foci above multiple papillae <25% of surface area, 3=extending over 25-50% of section, 4=>51% of section affected

**Ulceration:** 0=normal, 1=focus limited to region above a dermal papilla, 2=region over several hair follicles or foci above multiple papillae <25% of surface area, 3=extending over 25-50% of section, 4=>51% of section affected

**Crust formation:** 0=normal, 1= <25%, 2=26-50%, 3=51-75%, 4=76-100% of surface

**Epidermitis:** 0=normal, 1= <25%, 2=26-50%, 3=51-75%, 4=76-100% of surface

**Necrosis:** 0=normal, 1= <25%, 2=26-50%, 3=51-75%, 4=76-100% of surface

**Acantholysis:** 0=normal, 1=1-4, 2=5-8, 3=9-11, 4=>12 foci

**Spongiosis:** 0=normal, 1= <25%, 2=26-50%, 3=51-75%, 4=76-100% of epidermis

**Hydropic Degeneration:**  
0=absent, 1=present in scattered single cells, 2= present in scattered single cells and in clusters

**Orthokeratotic hyperkeratosis:**  
0=normal, 1= 1x normal, 2=2x normal, 3=3x normal, 4=>4x normal

**Parakeratosis:** 0=normal, 1=present (minimum/several layers w/nuclei), 2=present & >minimum

Hyperplasia: 0=normal, 1=2-3x normal, 2=4-5x normal, 3=6-7x normal, 4=>7x normal

Hypergranulosis:  
0=normal, 1=present

Inflammatory infiltrates:  
0=normal, 1=scattered single cells, 2=clusters of cells, 3=coalescing clusters,  
4=regionally diffuse infiltrates

Edema: 0=normal, 1=single focus within a dermal papilla, 2=several papillae, adjacent or  
separate w/ foci, 3=focus/foci filling papillae, 4=coalescing foci of edema

Vasodilation: 0=normal, 1=single, visible vessels, 2=several barely dilated vessels single or in cluster,  
3=clusters of moderately dilated vessels, 4=numerous widely dilated vessels

Direct quantitative observations for epidermal thickness are expressed in micrometers, and epidermal  
epithelial cell counts and counts of mitotic figures were made within the limitations stated on the  
assessment sheet.

## RESULTS AND COMMENTS

### Test Material Analysis

Results for the sample identified as POSF3404 applies to the JP-8 test substance (Table 3). Results for the JP-4 test material were not available, but will be reported in the definitive repeated dose skin irritation study on jet fuels.

**Table 3. Fuel Specification Results.**

Fuel	Method	Test	Result
POSF3404 (JP-8)	D1319	Aromatics, % vol	18
	D86	Distillation	
		IBP	159
		10 % recovered, °C	183
		20 % recovered, °C	190
		50 % recovered, °C	208
		90 % recovered, °C	246
		End Point, °C	266
		Residue, % vol	1.3
		Loss, % vol	1.1
	D56	Flash Point, °C	52
	D1298	Gravity, API	43.5
	D445	Viscosity @ -20°C, cSt	5.2
	D3343	Hydrogen Content, %wt	13.8
JP-4	D1319	Aromatics, % vol	
	D86	Distillation	
		IBP	
		10 % recovered, °C	
		20 % recovered, °C	
		50 % recovered, °C	
		90 % recovered, °C	
		End Point, °C	
		Residue, % vol	
		Loss, % vol	
	D56	Flash Point, °C	
	D1298	Gravity, API	
	D445	Viscosity @ -20°C, cSt	
	D3343	Hydrogen Content, %wt	

## **Addressing Initial Study Objectives**

The preliminary study began with defining the "dose." The criterion for dose selection was to simulate the amount (mass) of jet fuel on a human's hand (surface area) when "dipped" in jet fuel, i.e., the amount that would "wet" a person's hand. A dose of 2.5 g per 250 cm<sup>2</sup> was estimated (J. McDougal, T. Miller). An area of 2.5 cm x 5 cm (12.5 cm<sup>2</sup>) was chosen as an appropriate surface area for dosing laboratory rats. This area was measured on the dorsal side of the rat, using the midline as one of the 5 cm sides. To match the estimated "wet human hand" dose of 2.5 g/250 cm<sup>2</sup>, 0.156 mL test substance was calculated as the amount to be dispensed onto the 12.5 cm<sup>2</sup> surface area of the rat (specific gravity of 0.8 g/mL was used for both fuels).

Following careful hair clipping procedures, hair growth was observed daily. Though measurements were not made, investigators (D. Dodd, J. English) concluded that hair clipping on a weekly basis would allow adequate scoring of skin irritation, including the observation and recording of any lesions. Further, it did not appear that hair growth during a 7-day period would interfere with daily dermal application(s) of the test substance.

Normal (hand-held) animal restraint procedures were adequate for careful hair clipping and dosing of animals. Technicians worked in pairs. Study investigators concluded that additional procedures (e.g., tranquilization or anesthesia) were not necessary to carry out careful hair clipping and dosing procedures.

The criteria for stress associated with continuous wearing of neck collars were animal struggling, excessive scratching or pawing of the collar, increased activity, and increased responsiveness to noise or movement. Rats adapted to wearing the neck collars within a few days. On occasion, a collar was found to be "thrown off" by a rat. During clinical observation, body weighing, and dosing, collars were checked for fit (tightness without undue stress) and adjusted, if necessary.

## **Clinical Observations and Body Weights**

### **Dermal Application of JP-8 Twice Daily for 7 Days**

Slight erythema of the treated area was observed in all 4 rats during the first day of dosing. Erythema was well-defined throughout the remainder of the study. Slight edema was also observed. At necropsy, marked thickening and lichenification of the epidermis, with multiple dorsoventrally oriented cracks and full thickness fissures in the skin, were observed. Also, areas of skin sloughing and abundant serocellular crust were observed. Pre-dosing animal body weights ranged from 212 to 223 g. Animal activity appeared normal, though a mean weight gain of 2.5 g was observed between Study Day -2 (pre-dosing) and Study Day 8 (at necropsy). The mean weight gain of six untreated animals from the same animal shipment during the same observation period was 28.9 g. Note: These untreated animals were assigned to treatment groups at a later date.

### **Dermal Application of JP-8 Once Daily for 7 Days**

Slight erythema of the treated area was observed in 1 of 3 rats during the first day of dosing. Erythema was more well-defined throughout the remainder of the study, including the day of animal necropsy. No edema was observed. Study Day 1 (first day of dosing) animal body weights ranged from 260 to 273 g. A mean body weight gain of 7.8 g was observed between Study Day 1 and Study Day 7 (last day of dosing). This weight gain agrees with normal growth rate data of naïve male F-344 rats with body weights of approximately 260 to 270 g (Charles River Laboratories catalog).

#### **Dermal Application of JP-4 Twice Daily for 7 Days**

On Study Day 2 (second day of dosing), slight erythema of the treated area was observed in all 3 rats. The erythema progressed to moderate severity on Study Day 5. Slight edema was observed beginning on Study Day 3 (2 of 3 rats) or Study Day 4. The edema was more well-defined at the conclusion of the dosing period. Drying of the skin and lichenification, with multiple vertical splits in the epidermis was observed in all rats on Study Day 4 or 5. The severity of lichenification worsened throughout the remainder of the study. Study Day 1 body weights ranged from 225 to 247 g. Mean body weight gain from Study Day 1 to Study Day 8 (necropsy) was 6.8 g. Normal growth rate of naïve male F-344 rats with body weights of approximately 225 to 245 g is approximately twice this much during an 8-day interval (Charles River Laboratories catalog).

#### **Dermal Application of JP-4 Once Daily for 7 Days**

Slight erythema of the treated area was observed in 3 of 3 rats on the fourth through seventh days of dosing. No edema was observed. Study Day 1 animal body weights ranged from 255 to 259 g. A mean body weight gain of 9.8 g was observed between Study Day 1 (first day of dosing) and Study Day 7 (last day of dosing). This weight gain agrees with normal growth rate data of naïve male F-344 rats with body weights of approximately 255 to 260 g (Charles River Laboratories catalog).

### **Histopathology**

Average severity scores of treated and untreated rat skin are presented in the Table 4. The limited data were compared using t-test and a one-way analysis of variance (ANOVA) found in SigmaStat software, by Jandel Scientific.

**Table 4. Average Severity Scores of Combined Skin Lesions  
in Treated and Untreated Samples**

		Test Substance and Frequency of Application			
		JP-4		JP-8	
	Controls	1x/day	2x/day	1x/day	2x/day
Mean	19.2	64.6*	97.9*	86.8*	135.7*
St. Dev.	3.9	9.2	6.4	13.6	27.2

\*Significantly different from controls ( $P < 0.0001$ )

Mean scores for all treatment groups were found to be significantly higher than controls, with a P value less than 0.0001 for all four treatment groups using SigmaStat's t-test. The one-way ANOVA rendered similar results. Dermal response scores for JP-4 and JP-8 applied once daily were significantly lower than the level observed for the respective agent when applied twice daily, JP-4 ( $P = 0.0067$ ) and JP-8 ( $P = 0.0376$ ). Dermal responses to JP-4 applied once daily were not significantly different from responses to a once-a-day exposure to JP-8 ( $P = 0.0796$ ). Likewise, scores for twice daily applications of JP-4 and JP-8 did not differ significantly ( $P = 0.0692$ ) from one another.



The frequency of observed skin lesions is listed in Table 5.

**Table 5. Frequency of Skin Lesions in Treated and Untreated Samples**

Lesion Type	Controls	Test Substance and Frequency of Application			
		JP-4		JP-8	
		1x/day	2x/day	1x/day	2x/day
Erosion	0/5*	0/3	0/3	2/3	3/4
Ulceration	0/5	1/3	2/3	3/3	4/4
Crust Formation	0/5	2/3	3/3	2/3	4/4
Epidermitis	0/5	0/3	1/3	0/3	1/4
Necrosis	1/5	0/3	1/3	0/3	1/4
Acantholysis	0/5	0/3	2/3	0/3	3/4
Spongiosis	0/5	3/3	3/3	3/3	4/4
Hydropic Degeneration	5/5	3/3	3/3	3/3	3/4
Orthokeratosis	5/5	3/3	3/3	3/3	4/4
Parakeratosis	0/0	2/3	3/3	3/3	2/4
Hyperplasia	1/5	3/3	3/3	3/3	4/4
Hypergranulosis	0/5	3/3	3/3	3/3	4/4
Dyskeratosis	0/5	0/3	1/3	0/3	3/4
Inflammatory Infiltrates	0/5	0/3	1/3	2/3	3/4
Edema	0/5	0/3	2/3	0/3	4/4
Vasodilation	0/5	2/3	3/3	3/3	4/4

\*Number of samples with finding/number of samples examined

On the basis of lesion frequency, hydropic degeneration and orthokeratosis were changes consistently noted in all samples including controls and therefore do not serve as discriminating characteristics in this pilot study. However, considering mean severity scores as presented below, orthokeratosis does demonstrate an increase in the two groups receiving treatment twice-a-day.

Table 6 presents the mean severity scores for the skin lesions assessed. Epidermal thickness (as determined by direct measurement in 5 locations along the section and by counting and averaging the number of epidermal cells in another 5 locations) accounts for the greatest proportion of the dermal response score. For example, the average thickness of the epidermis in control animals was 13.5  $\mu$ m whereas, the thickness in the JP-8 1x/day and 2x/day was 61.6 and 90.5  $\mu$ m, respectively. Additionally, many other changes contribute in a minor way to overall score, and are directly related to epidermal thickness; for example, scores associated with proliferative changes (orthokeratosis, hyperplasia, and mitoses/4mm). Scores reflecting inflammatory change (i.e., inflammatory infiltrates, edema, and vasodilatation), although not directly associated with increased epidermal thickness did contribute appreciably to the cumulative score, as did degenerative changes (erosion, ulceration, and acantholysis). Proliferative and inflammatory changes were more commonly observed in the group receiving treatments twice daily.

**Table 6. Average Severity Scores of Skin Lesions in Treated and Untreated Samples**

Lesion Type	Controls	Test Substance and Frequency of Application			
		JP-4		JP-8	
		1x/day	2x/day	1x/day	2x/day
Erosion	0	0	0	0.67	1.0
Ulceration	0	0.33	1.33	1.33	3.00
Crust Formation	0	0.66	2.67	1.33	2.50
Epidermitis	0	0	0.33	0	0.25
Necrosis	0.20	0	0.67	0	0.50
Acantholysis	0	0	0.67	0	1.50
Spongiosis	0	1.00	1.33	1.33	2.25
Hydropic Degeneration	1.00	1.33	1.33	1.00	1.00
Orthokeratosis	1.20	1.00	2.00	1.67	3.00
Parakeratosis	0	0.67	1.33	1.00	0.50
Hyperplasia	0.20	1.00	2.33	2.00	3.25
Hypergranulosis	0	1.67	1.00	1.00	1.00
Dyskeratosis	0	0	0.33	0	0.75
Inflammatory Infiltrates	0	0	0.33	1.00	0.75
Edema	0	0	0.67	0	3.00
Vasodilation	0	1.00	1.00	1.33	3.25
Epidermal Thickness ( $\mu\text{m}$ )	13.52	45.87	64.93	61.60	90.50
Average Cells/100 $\mu\text{m}$	2.44	5.73	8.60	7.53	10.20
Mitoses per 4 mm	0.6	4.33	7.00	4.00	7.50

#### **Recommendations for Definitive Study**

1. To simulate human exposure scenario, the "dose" for the definitive study should be 0.156 mL test substance, applied to a 2.5 cm x 5 cm surface area of skin once daily (a.m.).
2. Hair clipping of the treated and untreated skin sites should be once-a-week. No clipping of hair on the day of an animal's termination.
3. Normal restraint procedures (hand-holding) should be adequate to carry out careful hair clipping procedures and dosing of animals.
4. Animals should wear neck collars to prevent licking or biting of treated and untreated skin sites.
5. Body weights should be determined to provide an indicator of health status.
6. Histopathology of treated and untreated skin would be the most valuable endpoint for determining, evaluating, and comparing skin irritancy and/or toxicity of test substances.
7. Histopathology of treated and untreated skin following recovery (no test substance application) would be another important endpoint to measure.

## REFERENCES

1. Bruner, RH. Pathological Processes of Skin Damage Related to Toxicant Exposure, pp 73-109, In: *Dermal and Ocular Toxicology: Fundamentals and Methods*. Ed. David W. Hobson, CRC Press, Inc., Boca Raton, FL, 1991.
2. Draize, J.H., Woodard, G., and Calvery, H.O., (1944). Method for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharmacol.* 82:377-390.
3. EPA Health Effects Testing Guidelines, section 798.4470, Primary dermal irritation. 40 CFR (7-1-90 edition)

## **APPENDIX A**

### **Individual Animal Histopathology Assessment Sheets**

(5 pages)



Group No.: 28 July 98				Test Article:	JP-4 2x/day
(Date of Necropsy)					1 week exposure
Animal No.	462-98 (4)	463-98 (14)	464-98 (28)		
<b>Epidermis</b>					
1. Erosion	0	0	0		
2. Ulceration	3	1	0		
3. Crust Formation	4	1	3		
4. Epidermitis	1	0	0		
	acute, focal				
5. Necrosis	2	0	0		
	N(MF)				
6. Acantholysis	1	0	1		
7. Spongiosis	1	1	2		
8. Hydropic Degeneration	1	1	2		
<b>Proliferative Changes</b>					
9. Orthokeratosis	2	2	2		
10. Parakeratosis	1	1	2		
11. Hyperplasia	3	2	2		
12. Hypergranulosis	1	1	1		
13. Dyskeratosis	1	0	0		
14. Epidermal Thickness ( $\mu\text{m}$ )	60.8	64.2	69.8		
(5 counts)	60/44/44/62/94	47/82/40/85/67	66/67/74/66/76		
15. Average Epi. Cells/100 $\mu\text{m}$	8.2	8.8	8.8		
(5 counts)	7/7/9/10/8	7/9/8/10/10	9/10/7/7/11		
16. Mitoses per 4 mm	7	5	9		
<b>Dermis</b>					
17. Inflammatory Infiltrates	0	1	0		
		acute, focal			
18. Edema	1	1	0		
19. Vasodilatation	1	1	1		
	several				
	intraepithelial				
	pustules				
Total Scores	99	91	103.6		
Group Average Score	97.9				

Group No.: 11 May 1998 (Date of Necropsy)				Test Article:	JP-8 neat 1x/day 1 week exposure
Animal No.	221-98 (5)	222-98 (6)	223-98 (7)		
<b>Epidermis</b>					
1. Erosion	1	0	1		
2. Ulceration	1	1	2		
3. Crust Formation	2	0	2		
4. Epidermitis	0	0	0		
5. Necrosis	0	0	0		
6. Acantholysis	0	0	0		
7. Spongiosis	1	1	2		
8. Hydropic Degeneration	1	1	1		
<b>Proliferative Changes</b>					
9. Orthokeratosis	2	1	2		
10. Parakeratosis	1	1	1		
11. Hyperplasia	2	2	2		
12. Hypergranulosis	1	1	1		
13. Dyskeratosis	0	0	0		
14. Epidermal Thickness ( $\mu$ m) (5 counts)	75.2 92/84/60/72/68	59.2 52/80/40/76/48	50.4 56/44/40/60/52		
15. Average Epi. Cells/100 $\mu$ m (5 counts)	8 8/9/8/6/9	7.8 6/9/7/11/6	6.8 8/6/7/6/7		
16. Mitoses per 4 mm	5	6	1		
<b>Dermis</b>					
17. Inflammatory Infiltrates (acute)	1 (acute)	0	2 (acute)		
18. Edema	0	0	0		
19. Vasodilatation	1	1	2		
Total Score	102.2	82	76.2		
Group Average Score	86.8				

Group No.: 29 April 1998				Test Article:	JP-8 neat, 2x/day
(Date of Necropsy)					1 week exposure
Animal No.	216-98 (1)	217-98 (2)	218-98 (3)	219-98 (4)	
<b>Epidermis</b>					
1. Erosion	1 (1 focus)	2 MF (2 foci)	1	0	
2. Ulceration	2 (1 focus)	3 MF (2 foci)	4	3	
3. Crust Formation	2	3	2	3	
4. Epidermitis	0	0	1 (w/folliculitis)	0	
5. Necrosis	0	0	0	2	
6. Acantholysis	2	2	2	0	
7. Spongiosis	2	3	2	2	
8. Hydropic Degeneration	1	2	0	1	
<b>Proliferative Changes</b>					
9. Orthokeratosis	3	3	3	3	
10. Parakeratosis	0	1	1	0	
11. Hyperplasia	3	3	3	4*	
12. Hypergranulosis	1	1	1	1	
13. Dyskeratosis	1 (MF single cell)	1 (single cell)	0	1 (MF)	
14. Epidermal Thickness ( $\mu\text{m}$ ) (5 counts)	62 80/60/40/50/80	122 60/100/90/100/260	84 120/70/40/60/130	94 80/140/120/60/70	
15. Average Epi. Cells/100 $\mu\text{m}$ (5 counts)	9.6 10/10/9/10/9	11.6 10/11/14/11/12	9.2 13/10/7/8/8	10.4 9/12/11/10/10	
16. Mitoses per 4 mm	7	5	12	6	
<b>Dermis</b>					
17. Inflammatory Infiltrates	1 (acute-very min.)	0	1 (acute folliculitis)	1 (acute-very min.)	
18. Edema	3	3	2	4	
19. Vasodilatation	3	4	2	4	
	intraepidermal hemorrhage	intraepidermal hemorrhage	subbasilar cleft	early epidermal vesicle	
	intraepidermal vesicles	intraepidermal vesicles			
*w/ minimal pseudocarcinomatous epithelial hyperplasia					
Total Score	103.6	169.6	130.2	139.4	
Group Average Score	135.7				



Group No.: 11 May 1998 (Date of Necropsy)				Test Article:		Controls		
Animal No.	226-98	225-98	224-98		223-98	222-98		
<b>Epidermis</b>								
1. Erosion	0	0	0		0	0		
2. Ulceration	0	0	0		0	0		
3. Crust Formation	0	0	0		0	0		
4. Epidermitis	0	0	0		0	0		
5. Necrosis	0	0	0		0	1		
6. Acantholysis	0	0	0		0	0		
7. Spongiosis	0	0	0		0	0		
8. Hydropic Degeneration	1	1	1		1	1		
<b>Proliferative Changes</b>								
9. Orthokeratosis	1	1	1		1	2		
10. Parakeratosis	0	0	0		0	0		
11. Hyperplasia	0	0	0		1	0		
12. Hypergranulosis	0	0	0		0	0		
13. Dyskeratosis	0	0	0		0	0		
14. Epidermal Thickness (um) (5 counts)	14.4 20/20/16/10/6	12.4 14/8/8/16/16	11.6 16/14/8/8/12		18.8 14/28/12/12/28	10.4 12/8/4/12/16		
15. Average Epi. Cells/100 um (5 counts)	2.4 3/3/3/2/1	2.4 3/3/1/2/3	2 3/2/2/1/2		3.4 3/4/2/3/5	2 2/1/1/3/3		
16. Mitoses per 4 mm	2	0	0		0	1		
<b>Dermis</b>								
17. Inflammatory Infiltrates	0	0	0		0	0		
18. Edema	0	0	0		0	0		
19. Vasodilatation	0	0	0		0	0		
Total Scores	20.8	16.8	15.6		25.2	17.4		
Group Average Score	19.2							